

CYTOLOGICAL OBSERVATIONS ON *PELOMYXA*
CAROLINENSIS WITH SPECIAL REFERENCE
TO THE MITOCHONDRIA

BY

REUBEN TORCH

B.S., University of Illinois, 1947

M.S., University of Illinois, 1948



AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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IN THE GRADUATE COLLEGE OF THE
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ACKNOWLEDGMENTS

I am indebted to Professor R. R. Kudo for his invaluable assistance throughout the course of this work. His helpful advice and constructive criticisms are gratefully acknowledged. I also wish to express my appreciation to the Graduate College of the University of Illinois, for granting me a research fellowship during the 1951-1952 academic year.

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In the present investigation, the mitochondria of *Pelomyxa carolinensis* have been studied as a step toward the determination of their possible significance within the cell. An attempt has been made to define their physical characteristics as accurately as possible by observing living unstained organisms, as well as vitally stained and fixed individuals. In addition, studies were made on the stratification of cytoplasmic inclusions within centrifuged animals, and a technique has been developed for the isolation of mitochondria by differential centrifugation.

Since 1944, clone cultures of the Illinois stock of *Pelomyxa carolinensis* have been maintained at the University of Illinois by Professor Kudo. Descendants of these clones have been used as the material for this study.

In living unstained organisms, the mitochondria are homogeneous, non-refractile bodies and display a diversity of shapes ranging from small spheres to very elongated rods. Dumbbell-shaped forms are also quite abundant. The rounded forms are most uniform in size and measure between 1.5-2.5 μ in diameter. Rod-shaped granules vary greatly in length from about 4 μ to 8 μ . Dumbbell-shaped forms are characteristically intermediate in size, measuring 4-6 μ in length. The granules are slightly amoeboid and are capable of altering their shape. They are incapable of independent locomotion, although they display pronounced Brownian movement.

The mitochondria are most conspicuously visible in areas immediately surrounding the contractile vacuoles. In optical section, there appears to be 2 or 3 layers of tightly packed granules around each contractile vacuole. Yet, if a surface or bottom view is obtained, there is definitely only a single layer present. As the vacuole is perfectly spherical and rotates

freely in the cytoplasm, the mitochondrial distribution observed in a surface view is probably indicative of the general distribution around the vacuole. Whenever a vacuole pulsates, the granular covering condenses and forms a compact mass around the minute opening which represents the systolic vacuole. As a rule, the granules adhere tenaciously to the vacuolar wall, and even though the vacuole may be carried rapidly through the cytoplasm, the mitochondria remain in their relative positions.

In addition to areas immediately surrounding the contractile vacuoles, mitochondria are also found in association with smaller food vacuoles. Vacuoles ranging in size from 10-30 μ usually are covered by a layer of granules in much the same manner as the contractile vacuoles. Mitochondria were never observed within food vacuoles, nor were they ever observed in intimate contact with large food vacuoles containing recently ingested paramecia.

Dumbbell forms and elongated rods occurring in certain other Protozoa have previously been thought to represent division stages. Continuous observation of these forms failed to reveal any actual division, and the question of mitochondrial division in *Pelomyxa carolinensis* remains undetermined.

Aqueous solutions of Janus green B, Janus blue, Janus black, Janus red, Rhodamine B, amethyst violet and neutral red were employed for vital staining of the organisms. Staining was accomplished in syracuse watch glasses containing 10 cc. of staining fluid. With the Janus dyes and amethyst violet, concentrations of 1:200,000 gave best results, while neutral red and Rhodamine B were employed at concentrations of 1:100,000 and 1:50,000 respectively.

Only Janus green B, Janus blue, and Janus red stain the mitochondria, and the reactions to the dyes are similar in all cases. Upon contact with the staining solution, the organisms form long, flattened, "mushroom-shaped" pseudopodia. This condition lasts for approximately 5 minutes, after which time the animals become monopodal in shape. The monopodal

stage is characterized by the presence of large anterior vacuoles which may measure 120-260 μ in diameter. Within 2 hours, most of the anterior vacuoles vanish, and the organisms resume active locomotion. The mitochondria are stained after 12 hours in Janus green B and Janus blue, and after 24 hours in Janus red. They appear as differentiated structures, with the stain localized in granules or crescentic areas located at eccentric positions within individual mitochondria.

Attempts to stain the mitochondria with the other dyes were unsuccessful. Janus black, amethyst violet and Rhodamine B color only the food vacuoles. In addition to neutral red bodies within vacuoles, neutral red stains all the cytoplasmic inclusions except mitochondria and fat. When organisms stained with neutral red are crushed, characteristic refractive caps form on the neutral red vacuoles. The caps measure 3-8 μ in diameter and appear in the form of concavo-convex discs, with the concave side proximal to the vacuole. In no case, were refractive caps seen in living organisms.

Sectioned material was prepared from organisms fixed in Champy's, Regaud's, Altmann's and Helly's fluids, as well as in a new "chrom-osmium" fixative. The latter is composed of 1% chromic acid and 2% osmium tetroxide in equal parts. Best results were obtained with Champy and chrom-osmium fixation, while Altmann's, Regaud's and Helly's fluids proved to be of little value. Fixed specimens were embedded in paraffin, sectioned at 6 μ , and stained with either Heidenhain's iron haematoxylin, acid aniline fuchsin, fast green, or according to Kull's fuchsin-toluidine blue-aurantia technique. The methyl green-pyronin technique was also employed in several instances. In chrom-osmium material, the mitochondria occur in a variety of shapes and sizes. Dumb-bell forms, short rods, and spheres are very abundant, but the extremely long rods seen in living cells are not present. The spheres measure 1.5-2.5 μ in diameter; the short rods are slightly larger, and the dumb-bell forms are mainly 3-4 μ in length. With the other fixatives, the mitochondria are invariably spherical in

shape and measure between 1.5-2.5 μ in diameter.

To determine the stratification of the various cytoplasmic inclusions, organisms were centrifuged at a force of 320 x gravity for 40 minutes. A 20% gum arabic solution was used as the suspension medium, and centrifugation was accomplished in hematocrit tubes constructed from 4 mm. soft glass tubing. Immediately after centrifuging, the animals were placed in ice water or in a cold chamber to prevent redistribution of the cytoplasmic components. The order of stratification from the centrifugal pole centripetally is: refractive bodies, food vacuoles, nuclei, crystals cast out of their vacuoles, crystals within vacuoles, empty crystal vacuoles, contractile vacuoles, and fat. The mitochondria occupy a stratum overlapping the food vacuoles and nuclei.

As a preliminary step toward obtaining qualitative data concerning their composition, mitochondria were isolated by differential centrifugation. After careful washing, the organisms were placed in a homogenizer and suspended in 10 times their volume of 0.1 M sucrose solution. The homogenizer consisted of a glass tube elongated at one end to form a nipple, the inside of which was ground. A long glass rod was ground at one end so that it formed a close fit within the nipple. Three minutes of vigorous stirring is sufficient to disrupt the majority of the organisms. The homogenate was centrifuged in 15 cc. plastic centrifuge tubes at 150 x gravity for 3 minutes. The resulting precipitate consisted of some whole animals, food vacuoles, refractive bodies, nuclei, and some mitochondria. Mitochondria and fat predominated in the supernatant, but constituents found in the precipitate were also present. This process was repeated 3 times, the precipitates discarded, and the supernatants preserved. The final supernatant was centrifuged at 3000 x gravity for one-half hour. A yellowish-brown precipitate resulted, which, under microscopic examination, was found to consist almost exclusively of mitochondria, although some bacteria were present.

In *Pelomyxa carolinensis*, the mitochondria possibly func-

tion in one or more of the following processes:

1. Accumulation and transportation of fluids.
2. Accumulation and transportation of waste products.
3. Digestion
4. Respiration

While there is some evidence for their participation in all these activities, their implication as respiratory centers is probably of greatest importance. Recent work on the chemistry of Janus green B staining shows that the coloration of the mitochondria is probably due to the presence of cytochrome oxidase within these structures. Similarly, biochemical studies on mitochondria of various Protozoa indicate the presence of respiratory enzymes as well as oxidation-reduction systems within these granules. While no definite conclusions can be drawn at the present time, the participation of protozoan mitochondria in cellular respiration is suggested. If such is the case, their accumulation in areas of high energy expenditure (contractile and food vacuoles) is understandable.

VITA

Reuben Torch was born in Chicago, Illinois, on December 20, 1926. After attending elementary school in Chicago, he graduated from Hyde Park High School in 1944 and entered the Massachusetts Institute of Technology under the auspices of the United States Navy. While a participant in the Navy V-12 program, he was transferred to Tufts College, Medford, Massachusetts in 1945. Soon after being discharged from the service in June, 1946, he enrolled at the University of Illinois, from which institution he received the degree of Bachelor of Science in 1947. At that time, he was admitted to the Graduate College of the University of Illinois, and was granted the degree of Master of Science in Zoology in 1948. In addition to his training at the University of Illinois, he spent the summer of 1949 at Hopkins Marine Station, Pacific Grove, California, where he completed a course in Invertebrate Zoology.

From 1947, he held a teaching assistantship in the Zoology department, interrupted only during 1951-1952, when he was awarded a University fellowship. In 1948 he was elected to active membership in Phi Sigma and associate membership in Sigma Xi. He is also a member of the Society of Protozoologists, the American Microscopical Society, and the American Association for the Advancement of Science.

